

Remarks

Election/Restrictions:

The Applicants affirm the election of Group 5, claims 53-64 and hereby cancel the remaining claims, claims 1-52 and 65-86. Via this amendment, Claim 61 is hereby cancelled, leaving claims 53-60, 62-64 and new claims 87-93 pending.

Drawings:

In response to the Examiner's objection to the Drawings as failing to include the appropriate references (i.e., Fig. 1, Fig. 2, or Fig. 3), marked up drawings showing the requested revision of the drawings and new, replacement drawings having those requested revisions were attached to the paper submitted on January 31, 2005.

Specification:

In response to the Examiner's objection to the Abstract, a revised abstract is presented on pages 2-3 of this paper.

In response to the Examiner's objection to the informality of inclusion of a hyperlink, revision of the Specification on page 4 of this paper is presented to remove the hyperlink.

The amendments of the abstract and specification contain no new matter.

Claims:

1. Rejection under 35 USC 112, second paragraph:

Claims 53-64 have been rejected for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

Via this Amendment, Claim 61 has been cancelled.

Specifically various aspects of claims 53, 62 and 63 are discussed in the Examiner's remarks and will be reviewed and commented upon herein below, particularly in view of the requested amendments of those claims.

a. The Examiner suggests a lack of antecedent basis for "said peptide extension" in Claim 53, correctly distinguishing the nucleic acid sequence encoding a peptide extension from the peptide extension encoded therein. Further, the Examiner correctly notes that it is the nucleic acids that are in-frame with one another, not the peptide extension and the nucleic acid encoding the protein or polypeptide of interest. The Applicants respectfully submit that Claim 53 as currently amended corrects these deficiencies.

b. The Examiner further notes that the "net negative charge ranging from -2 to -20" confers ambiguity and vagueness, particularly noting that no size is specified for the peptide extension and no conditions such as pH ranges are specified under which the net negative charge is determined. In addition, while in the Applicants' view it is not related to the potential ambiguity of the peptide extension having a net negative charge, the Examiner further notes that it is unclear whether there is any size limitation to the

polypeptide of interest and notes that there would be concerns as to whether the vector could accommodate nucleic acids for any size protein or polypeptide of interest.

To address the size of the peptide extension, a limitation of 61 or fewer amino acids, which is supported by the specification, has been entered into the amended Claim 53.

To address the issue of the conditions under which the encoded peptide extension has the requisite net negative charge, the amended Claim 53 includes the limitation that the net negative charge is relevant to physiological conditions. This limitation is supported by the specification since all expression is carried out *in vivo*, which is a physiological condition. Furthermore the specification includes notations of how the charges were calculated. See page 11, lines 5-8, for calculation of running net charge of encoded peptide extensions and polypeptides of interest and the legend to Table 1, page 45 indicating that, in addition, the amino and carboxyl termini of the peptides was used to calculate the charges of the various peptide extensions.

To address the question of accommodation of nucleic acid sequences encoding proteins or polypeptides of any size, the Applicants respectfully submit that, although this issue is not directly relevant to vectors offering enhancement of solubility and folding of encoded target proteins, the question of such accommodation has been explored throughout the history of use of recombinant DNA technologies to produce proteins. In fact, the present invention here, being an expression vector which, when used as it is intended for expression of a recombinant protein, fuses a negatively charged peptide extension to an encoded protein or polypeptide of interest, can be readily

incorporated into many different expression vectors that are specifically designed for expression of various sized proteins or polypeptides of interest. Those of ordinary skill in the art will have considerable experience in using various expression vectors and would have little, if any, difficulty in generating a modified expression vector which incorporated the ability to cause the negatively charged peptide extensions to be fused to the carboxyl-terminus of the protein or polypeptide of interest.

In summary of this section, the Applicants respectfully submit that Claim 53 as currently amended removes the potential ambiguity of the conditions for the claimed negative charge of the peptide extension and ordinary skill in the art is capable of addressing the issues surrounding the sizes of the proteins or polypeptides of interest that could be accommodated in the expression vector(s) of any kind, including those of the present invention.

The amendment of Claim 53 contains no new matter.

c. The Examiner notes that in claims 62 and 63 in the expression “activity promoting portions thereof” it is unclear how the term “activity” is to be interpreted. Although the specification suggests that “the activity” would be interpreted as whatever property it is within the sequence that promotes or enhances solubility and proper folding, e.g., see page 18, lines 14 through 20 where the negative charge of the peptide extensions is noted as providing sufficient repulsion to prevent aggregation:

“While not wishing to be bound by theory, it is thought that the strong repulsive force associated with the net negative charge of the peptide extension serves to segregate individual protein or polypeptide molecules following their release from the ribosome.”,

the current amendment of Claim 62 removes this phrase from the Claims.

Therefore, the amendment of Claim 62 contains no new matter.

In summary of the rejection of Claims 53-64 under 35 USC 112, second paragraph, the Applicants respectfully submit that the claims as amended are now in condition for allowance.

2. Rejection of Claims 62 –64 under 35 USC 112, first paragraph:

The Examiner notes that the originally filed genus claims 62 and 63 include the phrase “or activity promoting portions thereof”, causing the subject matter to be unsupported by the written description.

In response, as noted in the preceding section, the Applicants respectfully submit that Claims 62 and 63 as currently amended remove this notion and thus identify the inventive peptide extensions as comprising the carboxyl-terminal 57 amino acids of the T7 gene 10B protein, or substitution variants thereof. It is clear from the Markush group of dependent Claim 64 that the claimed peptides are supported by the written description in the specification. Thus, the Applicants submit that amended Claims 62-64 are now in compliance with 35 USC 112, first paragraph and are now in condition for allowance.

3. Rejection of Claims 53-64 under 35 USC 112, first paragraph:

The Examiner has rejected Claims 53-64 as being unsupported by the written description of the specification, noting that the specification provides insufficient guidance to enable a person skilled in the art to make and use the invention as broadly

claimed in the originally filed claims. The Applicants first note that Claim 61, via this Amendment, is cancelled and additionally respectfully submit that the written description of the specification clearly enables persons of skill in the art to make and use the claimed invention particularly as amended herein. Evidence to that is provided herein above and below. The following remarks are further supported by the Declaration of one of the Inventors of the present invention, which was included with the paper filed on January 31, 2005.

Scope/breadth of the claims: The Examiner notes that the originally filed claims:

“are directed to expression vectors comprising any peptide extension having the prescribed range of net negative charge, where the peptide extensions can be any size, linked to a protein of interest of any size, where the fusion proteins are expressed in any host, under any conditions (e.g. pH or temperature) and the extensions can have any “activity”.”

The currently amended claims address these issues in part and the methods of the specification address the other issues. The Applicants respectfully submit that the specification is clearly understandable by those of skill in the art.

First, the maximum length of the peptide extension is specified in the amended claim 53 and its dependent claims. In addition, the “activity” of the extensions is no longer an issue in amended Claims 62-64.

With respect to the size of the protein or polypeptide of interest, the Applicants submit that those of skill in the art will have sufficient experience to determine the likelihood that a potential protein or polypeptide of interest is or is not too large to have

a reasonable expectation of successful expression in a particular vector and/or in a particular expression system, i.e., whether the system is a bacterial, mammalian, insect, yeast or viral expression system or even if the system is an *in vitro* transcription-translation system. It is common knowledge that proteins and polypeptides of enormously varying sizes have been expressed by recombinant DNA methodologies. As this technology has now been in common practice for over 20 years the knowledge base and know-how of those of skill in the art is substantial.

Although it is most common that improper folding of recombinantly-expressed proteins occurs in prokaryotic (bacterial) systems where a substantial fraction of the cellular protein can be the expressed protein of interest, one of ordinary skill in the art will readily recognize that solubility- and proper folding-promoting peptide extensions would be expected to perform this function regardless of the host cell since all cellular and *in vitro* expression systems are “physiological”.

With respect to “any conditions”, it is highly unlikely that any person of ordinary skill in the art would try to express a protein in a cellular (or *in vitro*) expression system in conditions that were unfavorable for cell growth and normal metabolism. Thus, the Examiner is correct in suggesting that “any conditions” are appropriate, but only with the common knowledge that those “any conditions” must be compatible with cellular functioning and would thus need to be physiological.

Nature of the invention:

The Applicants wish to note that the Examiner has specifically and correctly identified the nature of the invention: “the peptide extension can be thought of as a solubilizing partner”. This is precisely the case.

State of the art/Unpredictability of the art:

The Examiner notes that Baneyx, F. suggests that the many “unknowns” about the fundamental aspects of the *E. coli* physiology “will affect progress in optimizing this bacterium for protein expression” implying that the direction of the “affect” will be a negative one. The Applicants respectfully draw the Examiner’s attention to the exact wording that is used by Baneyx:

“Recent advances in the understanding of the function, regulation and interactions of cellular gene products, together with the availability of new generic tools, **are making *E. coli* a more attractive host than ever** for the production of heterologous proteins. The facts that only a **small amount of information has been exploited** for practical purposes and that many fundamental aspects of *E. coli* physiology remain to be uncovered **will continue to fuel progress in optimizing this microorganism for protein expression.**” (emphasis added)

which shows that Baneyx predicts that continuing scientific exploration and the availability of new “generic tools” will continue to spur progress in the use of the bacterial expression systems. The present invention provides one such new tool that will aid in spurring such progress as it provides yet another “generic tool” i.e., the “solubilizing partner” for improving protein production.

The Examiner suggests that “vagaries as to protein production that often require considerable, costly and lengthy experimentation” will be needed to practice the present invention. As affirmed in the Inventor’s Declaration, the Applicants respectfully wish to

differ with respect to the difficulties involved in determining what expression system is best or optimal for use in preparing a protein or polypeptide of interest. In fact, this is not at all difficult or lengthy nor particularly costly today. There are a number of commercial organizations that provide tools for testing and using the various expression systems. As one example, Attachment A to this paper is a copy of the cover of the 2003-2004 Stratagene catalog and a copy of pages 83-104. These pages describe the various ready-to-use expression systems they have designed and “optimized” for use by those of ordinary skill in the art. The systems include bacterial (*E. coli*), yeast, mammalian, and viral vectors in addition to dual vectors (i.e., vectors for expression in either mammalian or bacterial cells). In addition to the bacterial and mammalian systems such as offered by Stratagene, Invitrogen further offers insect systems, both viral and vector-based, for expression of proteins. Because of the well-developed products offered by such research reagent suppliers, there is little concern that “vagaries” of one system would be a stumbling block to the use or trial of another system.

The Examiner also states that “attaching any sized peptide extension, fused to a target protein of any size would not necessarily result in expression or enhanced folding/solubility in any host organism”. The Applicants acknowledge that this is a well-accepted truth, although the claims as herein amended are directed **not** to “any sized peptide extension” but to negatively charged peptide extensions that are 61 or fewer amino acid residues.

The noted statements of Frydman are not relevant to the currently claimed invention as Frydman, in the cited statement, is discussing the difficulties in recovering proteins, particularly large or multi-domain proteins, from inclusion bodies:

“Unlike in vitro refolding of small single domain proteins, the refolding of larger proteins in vitro is generally inefficient or unsuccessful, particularly in the case of multidomain proteins.” (emphasis added)

The Examiner again raises the issue of the size of the target protein or polypeptide of interest and the Applicants again submit that those of ordinary skill in this art are accustomed to such “unknowns” when tackling the prospect of expressing a new target protein.

The Examiner raises the issue of vector loss from host cells and again the Applicants submit that those of ordinary skill in this art are well aware that this may be an issue and wish to point out that, as documented in the articles of the IDS and those cited by the Examiner, various strategies are available to the practitioners of this art for mitigating this problem. Furthermore, the Applicants very respectfully submit that this commonly noted problem is not directly relevant to the utility and practicability of the present invention.

The Examiner and, in particular, Banyex point out that “all fusion partners are not equally proficient at alleviating inclusion body formation” (see Banyex, p. 417, col 1). In fact the present invention supported by the specification includes this notion. The Applicants wish to draw the Examiner’s attention to Table 2, wherein the Applicants demonstrate that the solubility of some but not all of the randomly selected yeast proteins was enhanced by fusion with the T7B peptide. Further, the evidence in the

referenced table additionally clearly demonstrates that testing a peptide's solubility enhancing activity on a large number of target proteins is quite straightforward.

The Examiner notes that "even with proper folding, the fused protein may not contain native target protein activity, due to formation and isomerization of disulfide bonds . . .". The Applicants respectfully submit that "proper folding" normally implies the acquisition of a native protein activity, which would rarely be expected to occur if the disulfide bonds were improperly isomerized. However, the Applicants agree that native target protein activity is not always achieved when proteins are expressed as soluble entities in recombinant systems, and quite particularly when expressed as fusion proteins. It is a possibility that the fusion partner (particularly a large one, for example) could interfere with protein activity even in circumstances in which the fusion partner allowed the target protein to be soluble and generally properly folded. Again, the Applicants submit that the trial and error aspects of expressing new target proteins successfully is well-accepted and considered part of the state of the art and the Applicants and other practitioners make use of the widely available systems from the research reagent suppliers as noted above.

The Examiner also notes that protein expression is affected by the stability of the messenger RNA. The Applicants respectfully submit that, although RNA stability is not involved in protein solubility or proper folding and therefore not related to the utility or practicability of the present invention, RNA stability and instability is a well-accepted unpredictable aspect of expressing new target proteins. In fact, it is well-recognized that many very large proteins are difficult to express in bacterial systems simply and

particularly because messenger RNAs generally have such short half-lives in bacterial cells. Such being the case, many of skill in the art may attempt expression in a variety of cells to find the cell system (e.g., bacterial, yeast, mammalian, insect) that provides the best outcome. Again, the research reagent suppliers make such testing routine and simple.

The Examiner's comments that any one solubilizing partner does not "preclude inclusion body formation" were remarked upon herein above.

Amount of guidance provided:

The Examiner notes that:

"The specification does not provide any guidance as to what would be the size limitations for the peptide extensions, target proteins relative to particular vectors (i.e. vector loss/maintenance) or other cells whether eukaryotes or prokaryotes. Nor does the specification contemplate differentials in regard to temperature or pH, for example, in different cellular hosts, in effecting the charge characteristics for peptide extensions."

The Applicants again refer the Examiner to the above remarks wherein the ease with which testing of various host strains and vector systems is discussed. Experimentation upon initiating the production of new proteins or polypeptides of interest is an accepted aspect of this art and the commercial research reagent suppliers have facilitated the ease of this experimentation.

Number of working examples:

The Examiner suggests that the example target protein "CAR D1" is the only working example provided in the specification. The Applicants respectfully draw the Examiner's attention to Table 2 where 13 different proteins were examined as to

whether the T7B peptide enhanced solubility. The Table shows that the solubility of several proteins was enhanced at 37°C and at 25°C. The Table also shows the sizes of the tested proteins as ranging from 150 amino acids residues to almost 400 residues (394 residues – P40530). The Applicants further draw the Examiner's attention to page 36, line 19 through page 37, line 12 wherein the enhanced solubility of the over expressed E. coli protein ClpX was demonstrated. Thus, the specification contains several working examples.

Amount of experimentation required:

The Examiner suggests that the level of skill in the art “required to practice the claimed invention is high”. The Applicants respectfully draw the Examiner's attention to the remarks above as well as to Attachment B, affixed to this paper. Attachment B is a copy of the brochure for the Stratagene “VariFlex™ Bacterial Protein Expression System” in which the present invention is deployed. The system provides vectors of the present invention (and of inventions of claims that have been withdrawn from consideration through the restriction requirement). Such available research reagents enable the practice of the present invention by one of quite ordinary skill in the art. Please also note the statement on page 8 of the brochure “Since every protein is unique, the optimal SET tag needs to be determined empirically for each protein of interest. Stratagene therefore offers the SET-tagged vectors as complete sets, where vectors containing each of the three SET tag variants are provided.” (emphasis added) Thus, such research reagent suppliers facilitate and simplify the experimentation in this art. In addition, if the guidance provided in the specification of the present application was

insufficient to teach those of skill in the art how to make and use the present invention, these products would surely not be available in the marketplace today.

Claim rejection – 35 USC 102

4. Claims 53-61 have been rejected under 35 USC 102(b) as being anticipated by Rechsteiner et al. (US Patent No. 5,366,871), the '871 patent.

The Examiner states:

“The '871 patent teaches an expression vector to use in a cell where a peptide extension is linked to a gene of interest.” (emphasis added)

In response the Applicants, via this Amendment, have cancelled Claim 61 and further, respectfully submit for the Examiner's consideration a differing interpretation of the teachings wherein the '871 patent teaches **peptide extensions of interest, all of which are linked to the C-terminus (or a modified C-terminus) of a single protein, i.e., ubiquitin.** In the '871 teachings the peptide extensions fused to ubiquitin are then used as substrates for specific protein-modifying enzymes. For example, in Column 4, lines 17 through 53, various peptide extensions of interest are designated. They are SEQ ID NO: 1; SEQ ID NO: 3 and SEQ ID NO: 4, which serve as a protein kinase substrates and SEQ ID NO: 5 which serves as a substrate to examine enzymes that modify the HaRAS protein (e.g., farnesyl-protein transferase, carboxyl methyltransferase and etc.).

The Examiner additionally notes that:

“an intrinsic property for any peptide sequence in a solution is that at different pH values the peptide has a different net charge. Therefore, the

various peptides taught in the '871 patent could intrinsically contain a different net negative charge.”

The Applicants submit that despite the fact that peptides of SEQ ID NOs: 1, 3, 4, 7, and 8 have several Glu residues and therefore would have a net negative charge under physiological conditions, **they are all linked to ubiquitin, and not to various proteins or polypeptides of interest.** There are no teachings in the '871 patent that provide for enhancing the solubility and proper folding of expressed proteins or polypeptides of interest **since there are no proteins or polypeptides of interest.** Therefore the '871 patent cannot anticipate the present invention as claimed in the originally filed, as amended herein, and newly added claims.

The Examiner also states that:

“Expression is observed in prokaryotes (e.g. col. 9, Example 2) or it can also be in eukaryotes (e.g. col. 13, Example 10).”

The Applicants concur that in Example 2, the teachings show that a peptide-extended ubiquitin protein can be expressed in *E. coli*, but the Applicants respectfully wish to point out to the Examiner that the suggestion that “expression . . . can also be in eukaryotes” is a misinterpretation of teachings in the cited Examples and throughout the specification. Please review, for example, col. 2, lines 53 through 64, in particular noting that:

“Ubiquitin is an extremely soluble protein that can be expressed to very high levels within *E. coli* cells . . . Carboxyl terminal peptide extensions are easily and cheaply prepared **by cloning in *E. coli*.** For example, the preparation of **synthetic ubiquitin peptide fusion products containing up to about forty additional amino acid residues as ubiquitin extensions**

expressed in procaryotic cells, such as in E. coli, is described in
Rechsteiner et al., copending patent application . . .” (emphasis added)

And, further, please note that in Example 10 the teachings of the ‘871 patent show that in order to assay for kinases in eucaryotic cells the carboxyl terminus of ubiquitin was modified because:

“eucaryotic cells contain specific proteases for cleaving peptide extensions from ubiquitin . . . The existence of such enzymes presents a possible complication in the use of ubiquitin fusion peptides as kinase substrates, particularly when crude extracts are surveyed.” (col. 13, lines 58-63).

Thus, Rechsteiner et al, modified the carboxyl terminus of ubiquitin to remove the specifically-recognized cleavage site, then added peptide extensions and then determined whether or not:

“. . . Ub-AEX (SEQ ID NO: 4) extension, native ubiquitin, and Ub-PEST1 (SEQ ID NO: 2) **would serve as kinase substrate in crude extracts** from HeLa Cells, mouse liver or Xenopus eggs” (col. 14, lines 7-11). (emphasis added)

They did not express the carboxyl-modified ubiquitin-peptide extension fusion proteins in the eucaryotic cells, they merely tested crude extracts of the eucaryotic cells for kinase activity using the noted ubiquitin-peptide extension molecules that had been expressed in E. coli.

In conclusion, the Applicants submit that in view of the foregoing reasoning the ‘871 patent of Rechsteiner et al. does not and cannot anticipate the present invention.

5. Claims 53-54 and 56-63 are rejected under 35 USC 102(b) as being anticipated by Studier et al. (US 5,766,905), the ‘905 patent

The Examiner states:

“The ‘905 patent teaches expression vectors for expression of fusion proteins where a genes encoding a protein of interest are fused in-frame at the N-terminus of the 348 amino acid 10B protein (e.g. col. 3, ll 8-29). . . . As noted above, version of the 10B protein extension taught in the ‘905 patent would necessarily inhere a net charge depending on variability in pH. Thus at the appropriate pH, the 348 amino acid peptide extension can have a negative charge anywhere from -2 to -20.” (emphasis added)

The Applicants, via this Amendment have cancelled Claim 61, and also respectfully wish to draw the Examiner’s attention to the following teachings of the ‘905 patent at col. 2, line 66 through col. 3, line 7:

“In the initial experiments described in detail below, the T7 bacteriophage capsid protein was the selected structural protein. The capsid protein of bacteriophage T7 is normally made in two forms, 10A (344 amino acids) and 10B (397 amino acids), related by a well-defined translational frame-shift at amino acid position 341 (Condron et al., J. Bacterial 173:6998 (1991)). 10B is completely dispensable, which suggested that the **C-terminus** of 10B might be useful for phage display.” (emphasis added)

Thus, these statements indicate that a protein of interest is fused in-frame to the carboxyl terminus of the first 348 amino acid residues of the 10B protein (i.e., fused to the carboxyl end of the amino terminal 348 amino acid residues of the 10B protein), not fused “in-frame at the N-terminus” 10B protein. In the practice of the invention of the ‘905 patent, the gene 10B sequence was truncated so that it only encoded the first 348 amino acids of the 10B protein. Thus the sequences encoding the last (carboxyl terminal) 49 amino acids of the normal 10B protein are removed from these vectors. The sequences encoding the proteins or peptides of interest were then placed in-frame with the sequence encoding the first 348 amino acid residues of the 10B protein, thus replacing the sequences for the carboxyl-terminal 49 amino acid residues of the normal 10B protein. Thus the proteins or peptides to be displayed in the teachings of the ‘905

patent are fused to the 348th amino acid of the truncated 10B protein (i.e. fused to the carboxyl-terminal of the truncated 10B protein). And, resulting from this, the last 49 amino acids of the normal 10B protein are eliminated from the expressed fusion protein because the coding sequence for them is not present in the vector.

Thus the '905 patent cannot anticipate the present invention of the cited claims because in the invention of the '905 patent, the sequences encoding the carboxyl-terminal 49 amino acid residues of the normal 10B protein are no longer present in the "vectors", but are replaced with the sequences encoding the protein of interest. In contrast, in the claims of the present invention the sequences encoding the carboxyl-terminal 57, 40 or 18 amino acid residues of the 10B protein are present in the vector and when encoded are the peptide extensions and are not the protein of interest. Thus the '905 patent does not anticipate the present invention.

In addition, in the present invention a peptide extension of 61 or fewer amino acids is fused to the carboxyl terminus of the protein or polypeptide of interest so as to enhance the solubility and proper folding of the protein or polypeptide. There are no teachings in the '905 patent that suggest or imply or enable enhancing proper folding of proteins or polypeptides of interest. Thus, the '905 patent cannot anticipate the present invention.

6. Claims 53-61 rejected under 35 USC 102(b) as being anticipated by Harrison et al (US 5,989,868); see whole document; hereinafter the '868 patent).

The Applicants, via this Amendment, have cancelled Claim 61. In addition, the Examiner and the Applicants agree that the '868 patent teaches a vector for expression of fusion proteins. According to the specification, the '868 patent indicates that the protein of interest could be fused to either the carboxyl or amino terminus of the carrier protein (col. 9, ll 8-15). However, the drawing of Figure 2 contemplates a situation in which the protein of interest is fused at the carboxyl terminus of the carrier protein (peptide extension) and it is difficult to determine from the exemplifications whether all working examples were designed as shown in Figure 2.

The Applicants respectfully wish to point out that the '868 teachings include a prophetic suggestion that the contemplated system would be applicable for expression in cells other than bacterial cells and the Applicants draw the Examiners attention to column 3, lines 52 to 58:

“The selection of a carrier protein for the fusion protein which results in a more soluble fusion protein is based upon a revised version of the quantitative model developed by Wilkinson and Harrison (1991), for **prediction of the solubility of recombinant proteins expressed in *E. coli* at 37°C.** This model, which was based on data in the literature on **81 proteins express in *E. coli***” (emphasis added)

Because the model was based solely on solubility of proteins expressed in *E. coli*, it is questionable whether the specific formula for calculation of CV and the values for λ_1 and λ_2 and CV_{bar} are applicable to other cell types, particularly to non-bacterial cells such as yeast, insect or mammalian cells.

In addition, the Applicants wish to draw the Examiner's attention to column 9, lines 17 to 21:

"This invention is not limited to any specific type of heterologous protein. A wide variety of heterologous (i.e., foreign in reference to the host genome) genes or gene fragments are useful in forming the fusion sequences of the present invention." (emphasis added)

And, to column 15, lines 1 to 3 of Claim 1:

"1. A fusion gene comprising a first gene encoding a heterologous protein and a second gene encoding a carrier protein . . ." (emphasis added)

And to column 16, lines 1 to 2 of Claim 22:

"22. A method of producing a soluble heterologous protein comprising:" (emphasis added)

Thus the inventors of the '868 patent clearly contemplated the utility of this invention for the expression of fusion proteins in which the protein of interest was not native to the host cell. In the present case, however, it is demonstrated that the solubility-enhancing peptides of the present invention are applicable to expression of E. coli proteins in E. coli cells (see page 36, line 19 through page 37, line 12 wherein the enhanced solubility of ClpX was demonstrated) as well as applicable to expression of heterologous proteins (CAR-D1 (human) and the yeast proteins of Table 2).

In addition, the Applicants respectfully submit that the teachings of the '868 patent do not anticipate the invention of Claims 53-60, as herein amended, because, as the Examiner states, "the carrier proteins (peptide extensions) can be selected from a group of protein **varying in size from 146 to 495 amino acid residues** (e.g. col 4, Table 1)." It is clear from the specification of the '868 patent that the inventors did not

contemplate the use of carrier proteins (peptide extensions) that were shorter than 100 amino acids in length. Although no explanation as to why the inventors contemplated only carriers of more than 100 amino acid residues, the teachings of the '868 patent, do not suggest or imply, but substantially teach against a likely or reasonable expectation of success through the use of considerably shorter carrier proteins (such as the 61 or fewer amino acid peptide extensions of the present invention). In contrast, the solubilizing peptide extensions of the present invention are limited to peptide extensions of 61 or fewer amino acid residues.

Summary

This paper, originally filed in response to the Office Action mailed on August 24, 2004, is hereby filed with clarification of the status of Claim 61 (i.e., cancelled) in response to the Notice of Non-Compliant Amendment of April 19, 2005, includes the following: Amendments to the Abstract, Amendments to the Specification, Claim Listing and Remarks. The amendments of the Abstract, Specification and Claims contain no new matter. The additional materials (including marked up original drawings and replacement new drawings, Attachments A and B and the Declaration by one of the Inventors in further support of traversing the rejections), which were originally filed with the paper dated January 20, 2005, are not included with this paper.

Claims remaining under consideration include currently amended Claims 53, 56-60, and 62-64; originally filed Claims 54 and 55, and new Claims 87-93.

Appl. No. 10/037,243, Filed January 4, 2002
Amendment Dated April 25, 2005
Reply to Notice of Non-Compliant Amendment of April 19, 2005

In light of the above Amendments and Remarks, applicants respectfully submit that the instant application is now in condition for allowance and solicit a timely notice of allowance.

Respectfully submitted,



Christine L. Brakel
Agent for Applicants
Registration No. 45,772

Date: April 25, 2005

Christine L. Brakel
Patent Agent
Brookhaven National Laboratory
Bldg. 475D
P.O. Box 5000
Upton, New York 11973-5000
(631) 344-7134